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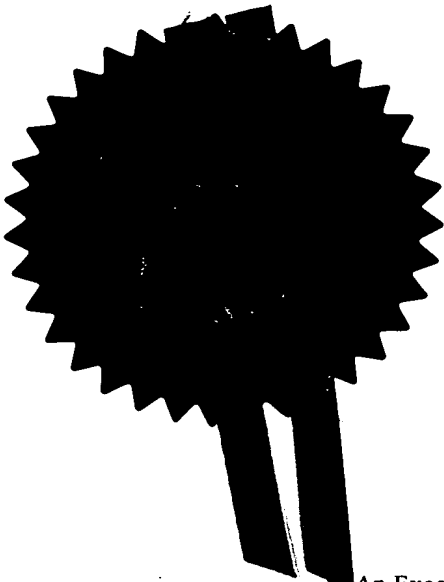
5

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1/77

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Request for grant of a patent

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22 APR 1998

1. Your reference

8.67993

2. Patent application number
(The Patent Office will fill in this part)

9808582.2

3. Full name, address and postcode of the
or of each applicant (underline all surnames)

NYCOMED IMAGING AS
Nycoveien 2
P.O. Box 4220 Torshov
N-0401 Oslo

Patents ADP number (if you know it)

If the applicant is a corporate body, give
country/state of incorporation

Norway

4. Title of the invention

Improvements in or relating to contrast
agents

5. Name of your agent (if you have one)

Frank B. Dehn & Co.

"Address for service" in the United Kingdom
to which all correspondence should be sent
(including the postcode)

179 Queen Victoria Street
London
EC4V 4EL

Patents ADP number (if you know it)

166001

6. If you are declaring priority from one or more
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and the date of filing of the or of each of these
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Country

Priority application number
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Date of filing
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7. If this application is divided or otherwise
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give the number and the filing date of
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Number of earlier application

Date of filing
(day / month / year)

8. Is a statement of inventorship and of right
to grant of a patent required in support of
this request? (Answer 'Yes' if:
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an
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Description 24

Claim(s) -

Abstract -

Drawing(s) -

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Priority documents -

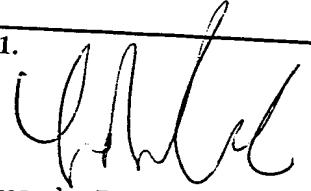
Translations of priority documents -

Statement of inventorship and right to grant of a patent (Patents Form 7/77) -

Request for preliminary examination and search (Patents Form 9/77) -

Request for substantive examination (Patents Form 10/77) -

Any other documents (please specify) -

11.  I/We request the grant of a patent on the basis of this application.

Signature

Date 22 April 1998

Frank B. Dehn & Co. - Agents for the applicant

12. Name and daytime telephone number of person to contact in the United Kingdom

J.C. Marsden
0171 206 0600

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Improvements in or relating to contrast agents

5 This invention relates to ultrasound imaging, more particularly to novel contrast agent preparations and their use in ultrasound imaging, for example in visualising tissue perfusion.

10 It is well known that contrast agents comprising dispersions of microbubbles of gases are particularly efficient backscatterers of ultrasound by virtue of the low density and ease of compressibility of the microbubbles. Such microbubble dispersions, if
15 appropriately stabilised, may permit highly effective ultrasound visualisation of, for example, the vascular system and tissue microvasculature, often at advantageously low doses.

20 The use of ultrasonography to measure blood perfusion (i.e. blood flow per unit of tissue mass) is of potential value in, for example, tumour detection, tumour tissue typically having different vascularity from healthy tissue, and studies of the myocardium, e.g. to detect myocardial infarctions. A problem with the application of existing ultrasound contrast agents to
25 cardiac perfusion studies is that the information content of images obtained is degraded by attenuation caused by contrast agent present in the ventricles of the heart.

30 In our copending but as yet unpublished International Patent Application No. PCT/GB97/02898, the contents of which are incorporated herein by reference, we have disclosed that ultrasonic visualisation of a subject, in particular of perfusion in the myocardium and other tissues, may be achieved and/or enhanced by
35 means of gas-containing contrast agent preparations which promote controllable and temporary growth of the gas phase *in vivo* following administration. Such

contrast agent preparations may be used to promote controllable and temporary retention of the gas phase, for example in the form of microbubbles, in tissue microvasculature, thereby enhancing the concentration of gas in such tissue and accordingly enhancing its echogenicity, e.g. relative to the blood pool.

Such use of gas as a deposited perfusion tracer differs markedly from existing proposals regarding intravenously administrable microbubble ultrasound contrast agents. Thus it is generally thought necessary to avoid microbubble growth since, if uncontrolled, this may lead to potentially hazardous tissue embolisation. Accordingly it may be necessary to limit the dose administered and/or to use gas mixtures with compositions selected so as to minimise bubble growth *in vivo* by inhibiting inward diffusion of blood gases into the microbubbles (see e.g. WO-A-9503835 and WO-A-9516467).

In accordance with PCT/GB97/02898, on the other hand, a composition comprising a dispersed gas phase is coadministered with a composition comprising at least one substance which has or is capable of generating a gas or vapour pressure *in vivo* sufficient to promote controllable growth of the said dispersed gas phase through inward diffusion thereto of molecules of gas or vapour derived from said substance. This latter substance is referred to as a "diffusible component", although it will be appreciated that transport mechanisms other than diffusion may additionally or alternatively be involved.

The preparations may advantageously be employed in visualising tissue perfusion in a subject, the increase in size of the dispersed gas being utilised to effect enrichment or temporary retention of gas in the microvasculature of such tissue, thereby enhancing its echogenicity.

The dispersed gas content of such contrast agent

preparations will tend to be temporarily retained in tissue in concentrations proportional to the regional rate of tissue perfusion. Accordingly, when using ultrasound imaging modalities such as conventional or harmonic B-mode imaging where the display is derived directly from return signal intensities, images of such tissue may be interpreted as perfusion maps in which the displayed signal intensity is a function of local perfusion. This is in contrast to images obtained using free-flowing contrast agents, where the regional concentration of contrast agent and corresponding return signal intensity depend on the actual blood content rather than the rate of perfusion of local tissue.

The present invention is based on the finding that growth of a dispersed gas in order to bring about its temporary retention in tissue microvasculature may be effected by administering the gas dispersion in conjunction with one or more substances serving to destabilise the dispersion. By avoiding use of a diffusible component requiring transport through blood and/or the continuous phase(s) in which the gas dispersion and diffusible component are contained, as well as through any encapsulating or other stabilising membranes which surround them, the invention may allow greater control of growth of the dispersed gas and may also involve a higher proportion of gas-containing structures in the growth process.

Thus according to one aspect of the present invention there is provided a combined preparation for simultaneous, separate or sequential use as a contrast agent in ultrasound imaging, said preparation comprising:

- i) an injectable gas dispersion, and
- ii) an administrable substance or substances capable of destabilising said dispersed gas so as at least transiently to increase the size thereof.

According to a further aspect of the invention

there is provided a method of generating enhanced images of a human or non-human animal subject which comprises the steps of:

5 i) injecting a physiologically acceptable aqueous medium having gas dispersed therein into the vascular system of said subject;

10 ii) before, during or after injection of said aqueous medium administering to said subject a substance or substances capable of destabilising said dispersed gas so as at least transiently to increase the size thereof; and

iii) generating an ultrasound image of at least a part of said subject.

15 In general any biocompatible gas may be present in the gas dispersion used in accordance with the invention, the term "gas" as used herein including any substances (including mixtures) at least partially, e.g. substantially or completely, in gaseous or vapour form at the normal human body temperature of 37°C.

20 Representative gases thus include air; nitrogen; oxygen; carbon dioxide; hydrogen; inert gases such as helium, argon, xenon or krypton; sulphur fluorides such as sulphur hexafluoride, disulphur decafluoride or trifluoromethylsulphur pentafluoride; selenium
25 hexafluoride; optionally halogenated silanes such as methylsilane or dimethylsilane; low molecular weight hydrocarbons (e.g. containing up to 7 carbon atoms), for example alkanes such as methane, ethane, a propane, a butane or a pentane, cycloalkanes such as cyclopropane,
30 cyclobutane or cyclopentane, alkenes such as ethylene, propene, propadiene or a butene, or alkynes such as acetylene or propyne; ethers such as dimethyl ether; ketones; esters; halogenated low molecular weight hydrocarbons (e.g. containing up to 7 carbon atoms); or
35 mixtures of any of the foregoing. Advantageously at least some of the halogen atoms in halogenated gases are fluorine atoms; thus biocompatible halogenated

hydrocarbon gases may, for example, be selected from bromochlorodifluoromethane, chlorodifluoromethane, dichlorodifluoromethane, bromotrifluoromethane, chlorotrifluoromethane, chloropentafluoroethane, 5 dichlorotetrafluoroethane, chlorotrifluoroethylene, fluoroethylene, ethylfluoride, 1,1-difluoroethane and perfluorocarbons. Representative perfluorocarbons include perfluoroalkanes such as perfluoromethane, perfluoroethane, perfluoropropanes or perfluorobutanes 10 (e.g. perfluoro-n-butane, optionally in admixture with other isomers such as perfluoro-iso-butane); perfluoroalkenes such as perfluoropropene, perfluorobutenes (e.g. perfluorobut-2-ene), perfluorobutadiene, perfluoropentenes (e.g. 15 perfluoropent-1-ene) or perfluoro-4-methylpent-2-ene; perfluoroalkynes such as perfluorobut-2-yne; and perfluorocycloalkanes such as perfluorocyclobutane, perfluoromethylcyclobutane, perfluorodimethylcyclobutanes, perfluorotrimethyl- 20 cyclobutanes, perfluorocyclopentane, perfluoromethylcyclopentane, perfluorodimethylcyclopentanes, perfluorocyclohexane, perfluoromethylcyclohexane or perfluorocycloheptane. Other halogenated gases include methyl chloride, fluorinated (e.g. perfluorinated) 25 ketones such as perfluoroacetone and fluorinated (e.g. perfluorinated) ethers such as perfluorodiethyl ether. The use of perfluorinated gases, for example sulphur hexafluoride and perfluorocarbons such as perfluoropropane, perfluorobutanes, perfluoropentanes 30 and perfluorohexanes, may be particularly advantageous in view of the recognised high stability in the bloodstream of microbubbles containing such gases. Other gases with physicochemical characteristics which cause them to form highly stable microbubbles in the 35 bloodstream may likewise be useful.

The dispersed gas may, for example, be present in the form of microbubbles at least partially encapsulated

or otherwise stabilised by gas-stabilising material.
This stabilising material may, for example, comprise a
coalescence-resistant surface membrane (for example
gelatin, e.g. as described in WO-A-8002365), a
5 filmogenic protein (for example an albumin such as human
serum albumin, e.g. as described in US-A-4718433, US-A-
4774958, US-A-4844882, EP-A-0359246, WO-A-9112823, WO-A-
9205806, WO-A-9217213, WO-A-9406477 or WO-A-9501187), a
10 polymer material (for example a synthetic biodegradable
polymer as described in EP-A-0398935, an elastic
interfacial synthetic polymer membrane as described in
EP-A-0458745, a microparticulate biodegradable
polyaldehyde as described in EP-A-0441468, a
15 microparticulate N-dicarboxylic acid derivative of a
polyamino acid - polycyclic imide as described in EP-A-
0458079, or a biodegradable polymer as described in WO-
A-9317718 or WO-A-9607434), a non-polymeric and non-
polymerisable wall-forming material (for example as
described in WO-A-9521631), or a surfactant (for example
20 a polyoxyethylene-polyoxypropylene block copolymer
surfactant such as a Pluronic, a polymer surfactant as
described in WO-A-9506518, or a film-forming surfactant
such as a phospholipid, e.g. as described in WO-A-
9211873, WO-A-9217212, WO-A-9222247, WO-A-9428780, WO-A-
25 9503835 or WO-A-9729783).

Where phospholipid-containing first compositions
are employed in accordance with the invention, e.g. in
the form of phospholipid-stabilised gas microbubbles,
representative examples of useful phospholipids include
30 lecithins (i.e. phosphatidylcholines), for example
natural lecithins such as egg yolk lecithin or soya bean
lecithin, semisynthetic (e.g. partially or fully
hydrogenated) lecithins and synthetic lecithins such as
dimyristoylphosphatidylcholine,
35 dipalmitoylphosphatidylcholine or
distearoylphosphatidylcholine; phosphatidic acids;
phosphatidylethanolamines; phosphatidylserines;

phosphatidylglycerols; phosphatidylinositols; cardiolipins; sphingomyelins; fluorinated analogues of any of the foregoing; mixtures of any of the foregoing and mixtures with other lipids such as cholesterol. The use of phospholipids predominantly (e.g. at least 75%) comprising molecules individually bearing net overall charge, for example as described in WO-A-9729783, may be particularly advantageous. Representative negatively charged phospholipids include naturally occurring (e.g. soya bean or egg yolk derived), semisynthetic (e.g. partially or fully hydrogenated) and synthetic phosphatidylserines, phosphatidylglycerols, phosphatidylinositols, phosphatidic acids and/or cardiolipins. Representative positively charged phospholipids include esters of phosphatidic acids such as dipalmitoylphosphatidic acid or distearoylphosphatidic acid with aminoalcohols such as hydroxyethylenediamine.

The disclosures of all of the above-described documents relating to gas-containing contrast agent formulations are incorporated herein by reference.

Gas microbubbles preferably have an initial average size not exceeding 10 μm (e.g. of 7 μm or less) in order to permit their free passage through the pulmonary system following administration, e.g. by intravenous injection. However, larger microbubbles may be employed where, for example, these contain a mixture of one or more relatively blood-soluble or otherwise diffusible gases such as air, oxygen, nitrogen or carbon dioxide with one or more substantially insoluble and non-diffusible gases such as perfluorocarbons. Outward diffusion of the soluble/diffusible gas content following administration will cause such microbubbles rapidly to shrink to a size which will be determined by the amount of insoluble/non-diffusible gas present and which may be selected to permit passage of the resulting microbubbles through the lung capillaries of the

pulmonary system.

The destabilising substance(s) (hereinafter referred to as "the destabiliser") employed to induce at least transient growth of the dispersed gas phase may operate by any of three mechanisms. Firstly the destabiliser may induce flocculation, aggregation or agglomeration whereby dispersed gas microbubbles assemble together to form clusters. This may be achieved by making the interbubble repulsive forces weaker than the interbubble attractive forces. The size of the resulting clusters may depend on factors such as the initial size and concentration of the microbubbles, the hydrodynamic behaviour of the surrounding blood stream, the ambient temperature and the net attractive force between the microbubbles.

Where the dispersed gas is stabilised by charge interactions, for example by interactions between charged surface groups in stabilising membranes of materials such as charged phospholipids, addition of charged species such as electrolytes will supply counter-ions which will screen the charged surface groups and reduce interbubble repulsive forces. The use of di- and multi-valent counter-ions is preferred; thus, for example, salts such as calcium chloride or magnesium chloride may be used to destabilise negatively charged membranes.

Aggregation may also be induced by modifying the properties of the surrounding water phase, for example by addition of solvents or of substances which change the electrical permeability of the medium so as to destabilise charge-stabilised gas dispersions. Representative examples of such substances include water-miscible liquids or solids, e.g. alcohols such as ethanol, isopropanol, ethylene glycol, propylene glycol, glycerol and sorbitol; aldehydes and ketones such as acetaldehyde and acetone; aliphatic esters such as methyl acetate, propyl formate and ethyl acetate; ethers

such as methyl propyl ether and di-isopropyl ether;
aliphatic amides such as N,N-dimethylformamide and
N,N-dimethylacetamide; aliphatic nitriles such as
acetonitrile; carbohydrates such as glucose and
5 sucrose; polyethylene glycol; polypropylene glycol etc.

Other representative destabilisers include
components capable of bridging dispersed gas
microbubbles. Examples of such components include
polymers, e.g. polysaccharides such as dextran and
10 starches; polyaminoacids such as polylysine and proteins
such as gelatin and albumin; polyethers such as
polyethylene glycol, polypropylene glycol and Pluronics;
polyvinyl pyrrolidone etc.

Aggregation may also be induced by adding
15 components which enter and modify the membrane, thereby
altering the forces between different microbubbles.
Examples of such compounds include fatty alcohols, fatty
acids and fatty amines; various surfactants; steroids
such as cholesterol etc.

20 Where the gas-stabilising material contains acid or
base groups on its surface, destabilisation may be
effected by addition of an appropriate acidic or basic
destabiliser in order to change the pH.

Aggregation may be a reversible or irreversible
25 process, depending on the magnitude of the forces
between the dispersed gas moieties. By optimising these
forces the aggregates may be made loose enough to be
easily disintegrated a short time after their temporary
retention in tissue microvasculature, thus ensuring that
30 the imaging procedure has a high safety profile. Such
disintegration or redispersion of the aggregates may if
desired be induced or enhanced by subsequent
administration of an appropriate substance. For
instance, a complexing agent such as ethylenediamine
35 tetraacetic acid may remove or neutralise the effect of
divalent or polyvalent ions used to destabilise charged
microbubbles. A buffer, acid or base may be used to

remove the effect of pH-induced aggregation/
destabilisation. Solvents, e.g. alcohols such as
ethanol, isopropanol, ethylene glycol, propylene glycol
and glycerol; aldehydes and ketones such as acetaldehyde
5 and acetone; ethers such as methyl propyl ether and di-
isopropyl ether; aliphatic amides such as N,N-
dimethylformamide and N,N-dimethylacetamide; or
aliphatic nitriles such as acetonitrile, may for example
be added to change the bridging behaviour of a polymeric
10 flocculant by altering the polymer's ability to coil in
the solvent. Such substances therefore re-establish the
repulsive forces between the dispersed gas moieties.

The second class of destabiliser useful in
accordance with the invention operates by inducing
15 coalescence or fusion of the dispersed gas moieties,
whereby two or more such moieties merge to create a
larger moiety. Such destabilisers may modify the
properties of gas-stabilising membrane material so that
membrane rupture between coalescing gas moieties is
20 enhanced. Representative destabilisers of this type
include surfactants, co-surfactants, solvents and other
substances which penetrate or otherwise modify the
properties of the stabilising membrane. Chemical
modification of the stabilising membrane, e.g. by
25 addition of electrolytes or by pH change, or chemical
reaction within the stabilising membrane may likewise be
used to induce flocculation.

The third class of destabiliser useful in
accordance with the invention comprises substances which
30 enhance Ostwald ripening of dispersed gas moieties. In
Ostwald ripening, larger microbubbles grow at the
expense of smaller ones, the driving force being the
higher pressure in small microbubbles compared to large
microbubbles as a result of differences in surface
35 tension in accordance with Laplace's law.

Growth of dispersed gas moieties by Ostwald
ripening may be initiated by increasing the solubility

of the gas in the surrounding aqueous phase, for example by adding a solvent or other appropriate component, e.g. a hydrotrope such as sodium benzoate or acetic acid, or a surfactant capable of solubilising the gas within micelles. Representative examples of solvents which may increase the solubility of the gas in the surrounding aqueous phase include aliphatic ethers such as ethyl methyl ether and methyl propyl ether; aliphatic esters such as methyl acetate, methyl formate and ethyl formate; aliphatic ketones such as acetone; aliphatic amides such as N,N-dimethylformamide and N,N-dimethylacetamide; and aliphatic nitriles such as acetonitrile. Representative examples of surfactants which may increase the solubility of the gas in the surrounding aqueous phase are those forming micelles in water, e.g. alkyl carboxylates, alkyl sulphonates, alkyl sulphates, dialkyl sulphosuccinates, alkylpyridinium salts, alkylammonium salts, alkyl polyethylene glycol ethers, alkyl polyethylene glycol esters and sorbitol fatty acid esters.

Ostwald ripening kinetics may also be enhanced by increasing the interfacial tension about the dispersed gas, for example by modifying the properties of stabilising membranes, e.g. through solvent addition, salt addition, pH change or addition of surfactants/ co-surfactants which provide higher surface tension.

As noted above, the destabiliser may be administered before, during or after injection of the gas dispersion; the order of administration and timing between them when they are separate may be varied to permit control over the time, rate and thus location of temporary retention of the dispersed gas.

In many instances the destabiliser will be administered by injection, e.g. intravenously, although intramuscular or subcutaneous injections may be useful in order to localise the effect of the destabiliser. Volatile destabilisers, e.g. solvents such as ethanol,

may, however, also be administered by inhalation; this may be of advantage in avoiding unwanted growth of the dispersed gas in the lung capillaries.

5 Imaging modalities which may be used in accordance
with the invention include two- and three-dimensional
imaging techniques such as B-mode imaging (for example
using the time-varying amplitude of the signal envelope
generated from the fundamental frequency of the emitted
10 ultrasound pulse, from sub-harmonics or higher harmonics
thereof or from sum or difference frequencies derived
from the emitted pulse and such harmonics, images
generated from the fundamental frequency or the second
harmonic thereof being preferred), colour Doppler
imaging, Doppler amplitude imaging and combinations of
15 these last two techniques with any of the other
modalities described above. To reduce the effects of
movement, successive images of tissues such as the heart
or kidney may be collected with the aid of suitable
synchronisation techniques (e.g. gating to the ECG or
20 respiratory movement of the subject). Measurement of
changes in resonance frequency or frequency absorption
which accompany growth of the dispersed gas may also
usefully be made to detect the contrast agent.

25 It will be appreciated that the dispersed gas
content of combined contrast agent preparations
according to the invention will tend to be temporarily
retained in tissue in concentrations proportional to the
regional rate of tissue perfusion. Accordingly, when
using ultrasound imaging modalities such as conventional
30 or harmonic B-mode imaging where the display is derived
directly from return signal intensities, images of such
tissue may be interpreted as perfusion maps in which the
displayed signal intensity is a function of local
perfusion. This is in contrast to images obtained using
35 free-flowing contrast agents, where the regional
concentration of contrast agent and corresponding return
signal intensity depend on the actual blood content

rather than the rate of perfusion of local tissue.

In cardiac studies, where perfusion maps are derived from return signal intensities in accordance with this embodiment of the invention, it may be
5 advantageous to subject a patient to physical or pharmacological stress in order to enhance the distinction, and thus the difference in image intensities, between normally perfused myocardium and any myocardial regions supplied by stenotic arteries.
10 As is known from radionuclide cardiac imaging, such stress induces vasodilatation and increased blood flow in healthy myocardial tissue, whereas blood flow in underperfused tissue supplied by a stenotic artery is substantially unchanged since the capacity for
15 arteriolar vasodilatation is already exhausted by inherent autoregulation seeking to increase the restricted blood flow.

The application of stress as physical exercise or pharmacologically by administration of adrenergic
20 agonists may cause discomfort such as chest pains in patient groups potentially suffering from heart disease, and it is therefore preferable to enhance the perfusion of healthy tissue by administration of a vasodilating drug, for example selected from adenosine, dipyridamole,
25 nitroglycerine, isosorbide mononitrate, prazosin, doxazosin, dihydralazine, hydralazine, sodium nitroprusside, pentoxifylline, amelodipine, felodipine, isradipine, nifedipine, nimodipine, verapamil, diltiazem and nitrous oxide. In the case of adenosine this may
30 lead to in excess of fourfold increases in coronary blood flow in healthy myocardial tissue, greatly increasing the uptake and temporary retention of contrast agents in accordance with the invention and thus significantly increasing the difference in return
35 signal intensities between normal and hypoperfused myocardial tissue. Because an essentially physical entrapment process is involved, retention of contrast

agents according to the invention is highly efficient; this may be compared to the uptake of radionuclide tracers such as thallium 201 and technetium sestamibi, which is limited by low contact time between tracer and tissue and so may require maintenance of vasodilatation for the whole period of blood pool distribution for the tracer (e.g. 4-6 minutes for thallium scintigraphy) to ensure optimum effect. The contrast agents of the invention, on the other hand, do not suffer such diffusion or transport limitations, and since their retention in myocardial tissue may also rapidly be terminated by the methods described above, the period of vasodilatation needed to achieve cardiac perfusion imaging in accordance with this embodiment of the invention may be very short, for example less than one minute. This will reduce the duration of any possible discomfort caused to patients by administration of vasodilator drugs.

In view of the fact that the required vasodilatation need only be short lasting, adenosine is a particularly useful vasodilating drug, being both an endogenous substance and having a very short-lasting action as evidenced by a blood pool half-life of only 2 seconds. Vasodilatation will accordingly be most intense in the heart, since the drug will tend to reach more distal tissues in less than pharmacologically active concentrations. It will be appreciated that because of this short half-life, repeated injection or infusion of adenosine may be necessary during cardiac imaging in accordance with this embodiment of the invention; by way of example, an initial administration of 150 $\mu\text{g/kg}$ of adenosine may be made substantially simultaneously with administration of the contrast agent composition, followed 10 seconds later by slow injection of a further 150 $\mu\text{g/kg}$ of adenosine, e.g. over a period of 20 seconds.

Contrast agent preparations in accordance with the

invention may advantageously be employed as delivery agents for bioactive moieties such as therapeutic drugs (i.e. agents having a beneficial effect on a specific disease in a living human or non-human animal), particularly to targeted sites. Thus, for example, therapeutic compounds may be present in the dispersed gas or may be linked to part of the stabilising material, e.g. through covalent or ionic bonds, if desired through a spacer arm.

10 The controllable growth properties of the dispersed gas may be utilised to bring about its temporary retention in the microvasculature of a target region of interest, and thus retention of the gas and associated therapeutic compound in a target structure is particularly advantageous.

15 The therapeutic compound, which may if desired be coupled to a site-specific vector having affinity for specific cells, structures or pathological sites, may be released as a result of, for example, stretching or fracture of the gas-stabilising material caused by growth of the dispersed gas, solubilisation of the stabilising material, or disintegration of gas-microbubbles or gas-containing microparticles. Where a therapeutic agent is chemically linked to the gas-stabilising material, the linkage or any spacer arm associated therewith may advantageously contain one or more labile groups which are cleavable to release the agent. Representative cleavable groups include amide, imide, imine, ester, anhydride, acetal, carbamate, carbonate, carbonate ester and disulphide groups which are biodegradable in vivo, e.g. as a result or hydrolytic and/or enzymatic action.

20 Representative and non-limiting examples of drugs useful in accordance with this embodiment of the invention include antineoplastic agents such as vincristine, vinblastine, vindesine, busulfan, chlorambucil, spiroplatin, cisplatin, carboplatin,

methotrexate, adriamycin, mitomycin, bleomycin, cytosine
arabinoside, arabinosyl adenine, mercaptopurine,
mitotane, procarbazine, dactinomycin (antinomycin D),
daunorubicin, doxorubicin hydrochloride, taxol,
5 plicamycin, aminoglutethimide, estramustine, flutamide,
leuprolide, megestrol acetate, tamoxifen, testolactone,
trilostane, amsacrine (m-AMSA), asparaginase (L-
asparaginase), etoposide, interferon a-2a and 2b, blood
products such as hematoporphyrins or derivatives of the
10 foregoing; biological response modifiers such as
muramylpeptides; antifungal agents such as
ketoconazole, nystatin, griseofulvin, flucytosine,
miconazole or amphotericin B; hormones or hormone
analogues such as growth hormone, melanocyte stimulating
15 hormone, estradiol, beclomethasone dipropionate,
betamethasone, cortisone acetate, dexamethasone,
flunisolide, hydrocortisone, methylprednisolone,
paramethasone acetate, prednisolone, prednisone,
triamcinolone or fludrocortisone acetate; vitamins such
20 as cyanocobalamin or retinoids; enzymes such as alkaline
phosphatase or manganese superoxide dismutase;
antiallergic agents such as amlexanox; anticoagulation
agents such as warfarin, phenprocoumon or heparin;
antithrombotic agents; circulatory drugs such as
25 propranolol; metabolic potentiators such as glutathione;
antituberculars such as p-aminosalicylic acid,
isoniazid, capreomycin sulfate, cycloserine, ethambutol,
ethionamide, pyrazinamide, rifampin or streptomycin
sulphate; antivirals such as acyclovir, amantadine,
30 azidothymidine, ribavirin or vidarabine; blood vessel
dilating agents such as diltiazem, nifedipine,
verapamil, erythritol tetranitrate, isosorbide
dinitrate, nitroglycerin or pentaerythritol
tetranitrate; antibiotics such as dapsone,
35 chloramphenicol, neomycin, cefaclor, cefadroxil,
cephalexin, cephradine, erythromycin, clindamycin,
lincomycin, amoxicillin, ampicillin, bacampicillin,

carbenicillin, dicloxacillin, cyclacillin,
picloxacillin, hetacillin, methicillin, nafcillin,
penicillin or tetracycline; antiinflammatories such as
diflunisal, ibuprofen, indomethacin, meclizine,
5 mefenamic acid, naproxen, phenylbutazone, piroxicam,
tolmetin, aspirin or salicylates; antiprotozoans such as
chloroquine, metronidazole, quinine or meglumine
antimonate; antirheumatics such as penicillamine;
narcotics such as paregoric; opiates such as codeine,
10 morphine or opium; cardiac glycosides such as
deslaneside, digitoxin, digoxin, digitalin or digitalis;
neuromuscular blockers such as atracurium mesylate,
gallamine triethiodide, hexafluorenum bromide,
metocurine iodide, pancuronium bromide, succinylcholine
15 chloride, tubocurarine chloride or vecuronium bromide;
sedatives such as amobarbital, amobarbital sodium,
apropbarbital, butabarbital sodium, chloral hydrate,
ethchlorvynol, ethinamate, flurazepam hydrochloride,
glutethimide, methotrimeprazine hydrochloride,
20 methypylon, midazolam hydrochloride, paraldehyde,
pentobarbital, secobarbital sodium, talbutal, temazepam
or triazolam; local anaesthetics such as bupivacaine,
chloroprocaine, etidocaine, lidocaine, mepivacaine,
procaine or tetracaine; general anaesthetics such as
25 droperidol, etomidate, fentanyl citrate with droperidol,
ketamine hydrochloride, methohexital sodium or
thiopental and pharmaceutically acceptable salts (e.g.
acid addition salts such as the hydrochloride or
hydrobromide or base salts such as sodium, calcium or
30 magnesium salts) or derivatives (e.g. acetates) thereof;
and radiochemicals, e.g. comprising beta-emitters. Of
particular importance are antithrombotic agents such as
vitamin K antagonists, heparin and agents with heparin-
like activity such as antithrombin III, dalteparin and
35 enoxaparin; blood platelet aggregation inhibitors such
as ticlopidine, aspirin, dipyridamole, iloprost and
abciximab; and thrombolytic enzymes such as

streptokinase and plasminogen activator. Other examples of therapeutics include genetic material such as nucleic acids, RNA, and DNA of natural or synthetic origin, including recombinant RNA and DNA. DNA encoding certain proteins may be used in the treatment of many different types of diseases. For example, tumour necrosis factor or interleukin-2 may be provided to treat advanced cancers; thymidine kinase may be provided to treat ovarian cancer or brain tumors; interleukin-2 may be provided to treat neuroblastoma, malignant melanoma or kidney cancer; and interleukin-4 may be provided to treat cancer.

Contrast agent preparations in accordance with the invention may be used as vehicles for contrast-enhancing moieties for imaging modalities other than ultrasound, for example X-ray, light imaging, magnetic resonance and, more preferably, scintigraphic imaging agents. Controlled growth of the dispersed gas phase may be used to position such agents in areas of interest within the bodies of subjects, which may then be imaged.

Contrast agent preparations in accordance with the invention may also be used as vehicles for therapeutically active substances which do not necessarily require release from the preparation in order to exhibit their therapeutic effect. Such preparations may, for example, incorporate radioactive atoms or ions such as beta-emitters which exhibit a localised radiation-emitting effect following growth of the dispersed gas phase and temporary retention of the agent at a target site. It will be appreciated that such agents should preferably be designed so that subsequent shrinkage and cessation of retention of the dispersed gas does not occur until the desired therapeutic radiation dosage has been administered.

Contrast agent preparations in accordance with the invention may additionally exhibit therapeutic properties in their own right. Thus, for example, the

dispersed gas may be targeted to capillaries leading to
tumours and may act as cell toxic agents by blocking
such capillaries. Concentrations of dispersed gas in
capillaries may also enhance absorption of ultrasonic
5 energy in hyperthermic therapy; this may be used in, for
example, treatment of liver tumours. Irradiation with a
relatively high energy (e.g. 5 W) focused ultrasound
beam, e.g. at 1.5 MHz, may be appropriate in such
applications.

10 The following non-limitative Examples serve to
illustrate the invention.

Preparation 1

Phosphatidylserine-stabilised perfluorobutane microbubble dispersion

5 Hydrogenated phosphatidylserine (5 mg/ml in a 1% w/w
solution of propylene glycol in purified water) and
perfluorobutane gas were homogenised in-line at 7800 rpm
and ca. 40°C to yield a creamy-white microbubble
10 dispersion. The dispersion was fractionated to
substantially remove undersized microbubbles (<2 µm) and
the volume of the dispersion was adjusted to the desired
microbubble concentration by adding aqueous sucrose to
15 give a sucrose concentration of 92 mg/ml. 2 ml portions
of the resulting dispersion were filled into 10 ml flat-
bottomed vials specially designed for lyophilisation,
and the contents were lyophilised to give a white porous
cake. The lyophilisation chamber was then filled with
perfluorobutane and the vials were sealed. Prior to
20 use, water was added to a vial and the contents were
gently hand-shaken for several seconds to give a
perfluorobutane microbubble dispersion.

Preparation 2

25 Phosphatidylserine-stabilised perfluorobutane
microbubble dispersion

30 Hydrogenated egg phosphatidylserine (50 mg) was
dispersed in water (9.75 ml, reversed osmosis quality)
containing propylene glycol (0.25 ml) by heating to 80°C
for about 2 minutes, followed by cooling to room
temperature. 1 ml portions of the dispersion were
transferred into ten 2 ml chromatography vials. The
35 headspaces were flushed with perfluorobutane gas and the
vials were capped and shaken on an Espe CapMix® shaker
for 45 seconds, yielding samples of a milky white

microbubble dispersion. The microbubble dispersions were collected in two 7 ml vials which were capped and placed on a roller table for two days. Later the samples were stored standing at room temperature until use.

Preparation 3

Sodium dodecyl sulphate solution

Sodium dodecyl sulphate (2.09 g) was added to water (7 ml, reversed osmosis quality) at room temperature to obtain a clear solution.

Preparation 4

Brij 99 solution

Brij 99 (2.84 g) was added to water (9.5 ml, reversed osmosis quality) and placed on a roller table overnight. A clear, slightly viscous solution was obtained.

Preparation 5

Sodium dodecyl sulphate/n-butanol/water microemulsion

Sodium dodecyl sulphate (12.5810 g), n-butanol (27.0813 g) and water (17.07 g, reversed osmosis quality) were mixed and diluted further with water to a water content of 30% w/w, yielding a clear, transparent microemulsion.

EXAMPLE 1

Microbubble aggregation by calcium chloride

One drop of the microbubble dispersion prepared in Preparation 1 was placed on an object glass for

microscopy investigation. The sample was covered with a cover glass and placed under a microscope. Droplets of a 50 mg/ml calcium chloride solution in water were added to the edge of the cover glass so that the solution penetrated into the microbubble dispersion. The behaviour of the microbubble dispersion as the calcium chloride solution front moved was recorded on a video tape. Microbubble aggregates with larger dimensions than the initial microbubbles were observed to form, demonstrating that microbubbles with potential for retention in capillary systems were generated.

EXAMPLE 2

Microbubble aggregation by sodium dodecyl sulphate

One drop of the microbubble dispersion prepared in Preparation 2 was placed on an object glass and covered by a cover glass. The sample was placed under a Zeiss Axioskop optical microscope equipped with a 10 x objective and a camera connected to a computer and an image analysis system. A droplet of the sodium dodecyl sulphate solution from Preparation 3 was placed on the edge of the coverglass, so that the solution penetrated under the coverglass and into the microbubble dispersion. The behaviour of the microbubble dispersion as the sodium dodecyl sulphate solution front moved was recorded, showing formation of microbubble aggregates with larger dimensions than the initial microbubbles, demonstrating the formation of microbubbles with potential for retention in capillary systems.

EXAMPLE 3

Microbubble aggregation by Brij 99

The procedure described in Example 2 was repeated using

the same microbubble dispersion from Preparation 2 but adding the Brij 99 surfactant solution from Preparation 4 to the edge of the coverglass. The experiment showed formation of microbubble aggregates with larger dimensions than the initial microbubbles, demonstrating the formation of microbubbles with potential for retention in capillary systems.

EXAMPLE 4

Microbubble growth by ethanol

The procedure described in Example 2 was repeated using the same microbubble dispersions from Preparation 2 but adding pure ethanol to the edge of the coverglass. The experiment showed growth of microbubbles resulting in microbubbles of larger size than the initial microbubbles, thereby demonstrating the formation of microbubbles with potential for retention in capillary systems.

EXAMPLE 5

Microbubble growth by isopropanol

The procedure described in Example 2 was repeated using the same microbubble dispersion from Preparation 2 but adding pure isopropanol to the edge of the coverglass. The experiment showed growth of microbubbles resulting in microbubbles of larger size than the initial microbubbles, thereby demonstrating the formation of microbubbles with potential for retention in capillary systems.

EXAMPLE 6

Microbubble growth by acetone

5 The procedure described in Example 2 was repeated using
the same microbubble dispersion from Preparation 2 but
adding pure acetone to the edge of the coverglass. The
experiment showed growth of microbubbles resulting in
microbubbles of larger size than the initial
10 microbubbles, thereby demonstrating the formation of
microbubbles with potential for retention in capillary
systems.

EXAMPLE 7

15

Microbubble aggregation and growth by a sodium dodecyl
sulphate/n-butanol/water microemulsion

One droplet of the microbubble dispersion from
20 Preparation 2, diluted three times with water was placed
on an object glass and covered by a coverglass. The
sample was placed under a Zeiss Axioskop optical
microscope equipped with a 40 x objective and a camera
connected to a computer and an image analysis system. A
25 droplet of the sodium dodecyl sulphate/n-butanol/water
microemulsion from Preparation 5 was placed on the edge
of the coverglass, so that the microemulsion penetrated
under the coverglass and into the microbubble
dispersion. The behaviour of the microbubble dispersion
30 as the microemulsion front advanced was recorded,
showing formation of microbubble aggregates with larger
dimensions than the initial microbubbles. A short time
afterwards, the microbubbles in the aggregates fused or
coalesced to give larger microbubbles, demonstrating the
35 formation of microbubbles with potential for retention
in capillary systems.

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